AssayMax^{TM}

Human Complement C4BP ELISA Kit

Assaypro LLC
3400 Harry S Truman Blvd
St. Charles, MO 63301
T (636) 447-9175
F (636) 395-7419
www.assaypro.com

For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

Thank You for choosing Assaypro

Assay Summary

Step 1. Add 50 µl of Standard or Sample per well.
Incubate 2 hours.

Step 2. Wash, then add 50 µl of Biotinylated Antibody per well.
Incubate 1 hour.

Step 3. Wash, then add 50 µl of SP Conjugate per well.
Incubate 30 minutes.

Step 4. Wash, then add 50 µl of Chromogen Substrate per well.
Incubate 15 minutes.

Step 5. Add 50 µl of Stop Solution per well.
Read at 450 nm immediately.

Symbol Key

Consult instructions for use.

Assay Template

	A	B	C	D	E	F	G	H
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Human Complement C4b-Binding Protein (C4BP) ELISA Kit

Catalog No. EC2202-1

Sample protocol for reference use only

Introduction

Complement component C4b-binding protein (C4BP) regulates the complement system by accelerating the decay of the complement component C3 convertase and by acting as a cofactor to the serine protease factor I in the degradation of C4b (1). C4BP is a high molecular mass (570 kDa) glycoprotein and is present in plasma in various isoforms with different alpha beta composition. The major form of C4BP is composed of seven identical 70-kDa alpha chains, each containing a binding site for the complement protein C4b, and a unique 45-kDa beta chain which contains a binding site for the vitamin K-dependent protein S (2). C4BP was overexpressed in the synovial membranes of patients with rheumatoid arthritis (3). It was detected in amyloid-beta plaques and on apoptotic cells in Alzheimer's disease brain (4). C4BP could directly bind to ovarian tumor cell lines and was associated with non-small cell lung cancer (5, 6).

Principle of the Assay

The AssayMax Human Complement C4BP ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human complement C4BP in **plasma, serum, urine, milk, saliva, CSF, and cell culture samples**. This assay employs a quantitative **sandwich enzyme immunoassay** technique that measures human complement C4BP in less than 4 hours. A polyclonal antibody specific for human complement C4BP has been pre-coated onto a 96-well microplate with removable strips. Complement C4BP in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for complement C4BP, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- Prepare all reagents (working diluent buffer, wash buffer, standard, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.

- Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.
- Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents.
- The Stop Solution is an acidic solution.
- This kit is for research use only.
- The kit should not be used beyond the expiration date.

Reagents

- **Human Complement C4BP Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human complement C4BP.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human Complement C4BP Standard:** Human complement C4BP in a buffered protein base (60 ng, lyophilized).
- **Biotinylated Human Complement C4BP Antibody (50x):** A 50-fold concentrated biotinylated polyclonal antibody against complement C4BP (140 µl).
- **MIX Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x)**: A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 µl).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store SP Conjugate and Biotinylated Antibody at -20°C.
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- Diluent (1x) may be stored for up to 30 days at 2-8°C.

- Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µl, 20-200 µl, 200-1000 µl, and multiple channel).
- Deionized or distilled reagent grade water.

Sample Collection, Preparation, and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x *g* for 10 minutes. Dilute samples 1:40000 into MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes and remove serum. Dilute samples 1:40000 into MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Urine:** Collect urine using sample pot. Centrifuge samples at 800 x *g* for 10 minutes. Dilute samples 1:2 into MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Saliva:** Collect saliva using sample pot. Centrifuge samples at 800 x *g* for 10 minutes. Dilute samples 1:80 into MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 3000 x *g* for 10 minutes to remove debris. Collect supernatants and assay. Samples can be stored at -20°C or below. Avoid repeated freeze-thaw cycles.
- **Milk:** Collect milk using sample tube. Centrifuge samples at 800 x *g* for 10 minutes and assay. Milk dilution is suggested at 1:800 into MIX Diluent; however, the user should determine the optimal dilution factor. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **CSF:** Collect cerebrospinal fluid (CSF) using sample pot. Centrifuge samples at 3000 x *g* for 10 minutes. Dilute samples 1:20 into MIX Diluent and assay. The undiluted samples can be stored at -80°C for up to 3 months. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **MIX Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- **Standard Curve:** Reconstitute the 60 ng of Human Complement C4BP Standard with 1 ml of MIX Diluent to generate a 60 ng/ml standard stock solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard stock solution (60 ng/ml) 1:2 with MIX Diluent to produce 30, 15, 7.5, 3.75, 1.875, and 0.938 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[C4BP] (ng/ml)
P1	1 part Standard (60 ng/ml)	60.00
P2	1 part P1 + 1 part MIX Diluent	30.00
P3	1 part P2 + 1 part MIX Diluent	15.00
P4	1 part P3 + 1 part MIX Diluent	7.500
P5	1 part P4 + 1 part MIX Diluent	3.750
P6	1 part P5 + 1 part MIX Diluent	1.875
P7	1 part P6 + 1 part MIX Diluent	0.938
P8	MIX Diluent	0.000

- **Biotinylated Human Complement C4BP Antibody (50x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with MIX Diluent. Any remaining solution should be frozen at -20°C.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.

Assay Procedure

- Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).

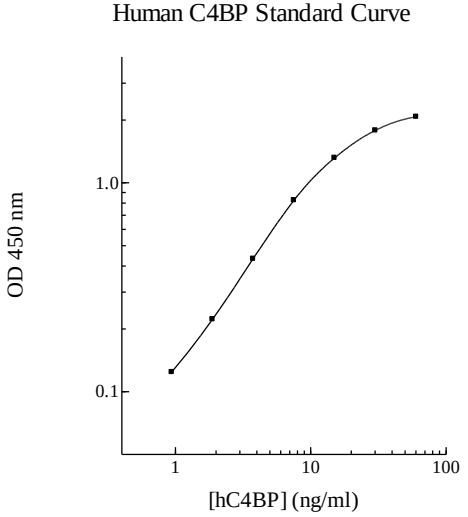
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Human Complement C4BP Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
- Add 50 µl of Biotinylated Human Complement C4BP Antibody to each well and incubate for 1 hour.
- Wash the microplate as described above.
- Add 50 µl of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.
- Add 50 µl of Chromogen Substrate per well and incubate for 15 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on the microplate reader at a wavelength of 450 nm **immediately**. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using four-parameter or log-log logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Performance Characteristics

- The minimum detectable dose of complement C4BP is typically ~ 0.9 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 5.0% and 7.1% respectively.

Linearity

Average Percentage of Expected Value (%)		
Sample Dilution	Plasma	Serum
1:20000	90%	91%
1:40000	98%	98%
1:80000	107%	106%

Average Percentage of Expected Value (%)	
Sample Dilution	Milk
1:400	92%
1:800	99%
1:1600	105%

Recovery

Standard Added Value	1.87 – 30 ng/ml
Recovery %	85 – 111%
Average Recovery %	97%

Cross-Reactivity

Species	**Cross Reactivity (%)**
Monkey	None
Mouse	None
Rat	None
Swine	None
Canine	None
Bovine	None
Human	100%
Proteins	**Cross Reactivity (%)**
Complement C1	None
Complement C3	None
Complement C4BP	100%
Complement C4	None
Complement C5	None
Complement C6	None
Complement C7	None
Complement C8	None
Complement C9	None

Reference Value

- On average, normal human C4BP plasma level is 200 µg/ml.

References

(1) Gigli I *et al.* (1979) *Proc. Natl. Acad. Sci. USA* 76: 6596–6600
(2) Hillarp A and Dahlbäck B (1990) *Proc. Natl. Acad. Sci. USA*. 87(3):1183-1187
(3) Sánchez-Pernaute O *et al.* (2006) *Ann Rheum Dis*. 65(10):1279-1285
(4) Trouw LA *et al*. (2008) *Mol Immunol*. 45(13):3649-3660
(5) Holmberg MT *et al.* (2001) *J Immunol.* 167(2):935-939
(6) Okroj M *et al.* (2008) *Mol Immunol.* 45(1):169-179

Version 1.0

Related products

- EC1102-1 AssayMax Human Complement C1r ELISA Kit (Plasma, Serum, Urine, Milk, Saliva, and Cell Culture samples)
- EC1101-1 AssayMax Human Complement C1q ELISA Kit (Plasma, Serum, Urine, Milk, Saliva, and Cell Culture samples)
- EC1111-1 AssayMax Human Complement C1 ELISA Kit (Plasma, Serum, Urine, Milk, Saliva, and Cell Culture samples)
- EC2001-1 AssayMax Human Complement C2 ELISA Kit (Plasma, Serum, Saliva, and Cell Culture samples)
- EC2101-1 AssayMax Human Complement C3 ELISA Kit (Plasma and Serum samples)
- EC3201-1 AssayMax Human Complement C3 ELISA Kit (Urine, Milk, Saliva, and Cell Culture samples)
- EC3301-1 AssayMax Human Complement C3b ELISA Kit (Plasma, Serum, Urine, Milk, Saliva, and Cell Culture samples)
- EC2102-1 AssayMax Human Complement C4 ELISA Kit (Plasma and Serum samples)
- EC3202-1 AssayMax Human Complement C4 ELISA Kit (Urine, Milk, Saliva, and Cell Culture samples)
- EC5101-1 AssayMax Human Complement C5 ELISA Kit (Plasma, Serum, Milk, Saliva, and Cell Culture samples)
- EC6101-1 AssayMax Human Complement C6 ELISA Kit (Plasma, Serum, Urine, Saliva, Milk, and Cell Culture samples)
- EC7101-1 AssayMax Human Complement C7 ELISA Kit (Plasma, Serum, Urine, Milk, Saliva, and Cell Culture samples)
- EC8101-1 AssayMax Human Complement C8 ELISA Kit (Plasma, Serum, Urine, Milk, Saliva, and Cell Culture samples)
- EC9101-1 AssayMax Human Complement C9 ELISA Kit (Plasma, Serum, Urine, Milk, Saliva, and Cell Culture samples)